Genetic engineering

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Phage genome engineering with retrons

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A method using retron recombineering for phage genome modification may facilitate access to therapeutic phages.

Phage therapy, which harnesses bacterial viruses (phages) to treat pathogenic bacterial infections, has long been suggested as an alternative to antibiotics. Natural phages were previously successfully used to treat patients with antibiotic-resistant bacterial infections[1], but converting natural phages into effective antimicrobial drugs often requires genome engineering to increase their efficiency[2] or to remove undesired traits[3]. Current techniques for editing phage genomes are labor intensive and inefficient, posing a hurdle to phage therapy innovation. In a new *Nature Biotechnology* paper, Fishman et al.[4] present a system that makes phage modification simpler and more scalable. Their method allows multiple targeted edits in phage genomes with high accuracy and in only a few hours of hands-on time. This easy and rapid approach to phage genome editing may improve the development of therapeutic phages in the fight against antimicrobial-resistant pathogens.

Most of the current methods used to modify phage genomes rely on homologous recombination. This process requires the introduction of 'donor' DNA that is homologous to a specific region in the phage genome but contains the desired modifications. When a phage infects a bacterium that contains donor DNA (usually introduced on a plasmid), homologous recombination replaces a part of the phage DNA with donor DNA, generating a modified phage. Since this event is rare, it is necessary to establish conditions that will eliminate unmodified phages, a process called counterselection. CRISPR-based counterselection uses Cas9 to cleave unmodified phage genomes or Cas13 to kill cells that express RNA from unmodified phages[5]. However, one of the main limitations of this procedure is that it does not scale well. If multiple changes in the same phage genome are necessary, counterselection must be tailored for each change individually, making it a lengthy and labor-intensive process.

Modified phages can also be obtained using fully synthetic approaches. Multiple mutations can be introduced in a phage genome at once by synthesizing the modified phage DNA ex vivo and then transforming the DNA into bacterial cells or adding it to cell-free transcription and translation reactions. This process, called phage 'rebooting', is expensive and inefficient, especially for phages with large genomes[5].

In the new study, Fishman et al. introduce a system for phage genome engineering that improves on these methods. Their method can produce modifications at multiple sites in a phage genome rapidly, efficiently, and without the need for counterselection. To this end, the authors use retrons as an alternative source of donor DNA for homologous recombination. Retrons are bacterial retroelements that naturally defend bacteria against phage infection[6]. These elements consist of a noncoding RNA and a reverse transcriptase[7]. The reverse transcriptase uses a specific region in the noncoding RNA as a template to synthesize a single-stranded DNA. By replacing the noncoding RNA template with a sequence containing a desired edit, retrons can be used to produce donor DNA templates for genome engineering[8]. To optimize retrons for phage genome modification, Fishman et al. combine the expression of a modified retron with a single-stranded annealing protein that ensures efficient annealing of the donor DNA to the lagging strand of the replication fork in the phage DNA. As a result, the retron-generated donor DNA is incorporated into the newly replicated genome (Fig. 1).

The genome engineering tool, called phage retron recombineering, allows several kinds of genetic manipulations, including point mutations, deletions of up to 300 bp and insertions of up to 30 bp. Wild-type phages infecting bacteria that express a modified retron, called a recombitron, are edited efficiently, and repeating the process by infecting a fresh culture of recombitron-expressing cells with progeny phages increases the proportion of edited phages in each round. The authors show that editing rates can be further improved using a modified host strain that optimizes recombination conditions. Overall, after three rounds of culture, they report >95% editing rates for lambda, 30% for T7 and 5% for T5 phage. The high proportion of edited phages makes counterselection unnecessary, which is a major benefit of this approach.

A key advantage of phage retron recombineering is that it can install several modifications in a single experiment. To achieve multiplexed editing, the authors mix multiple bacterial strains, each expressing a distinct recombitron targeting a different locus in the phage genome, and then propagate phages through these cultures. In these conditions, each new infection event is an opportunity to acquire an edit from one of the recombitrons. The authors show that after three rounds of propagation on fresh cultures, which can be performed in 3 days and with less than 2 hours of hands-on time, up to five edits can accumulate in a single phage.

Fishman et al. demonstrate the effectiveness of multiplexed editing by introducing multiple modifications in the tip domain of the phage T7 tail fiber. This domain is involved in recognizing the lipopolysaccharide of the bacterial host, and bacteria mutated in genes responsible for building the lipopolysaccharide are notoriously resistant to phage infection. The authors used eight distinct recombitrons, each designed to cause a different tail tip mutation that was previously shown to enable T7 replication on a lipopolysaccharide-mutated host. With this mixture, they generated several dozen phages with combinatorial mutations, identifying particular combinations that enhance phage infection properties toward mutated hosts.

Phage retron recombineering is not without limitations. One limitation is the low efficiency of editing phages with modified DNA, such as T2 and T4. Sampling the thousands of retrons naturally occurring in bacterial genomes might lead to the discovery of retrons with an improved capacity to edit phages with modified DNA. Testing recombitrons in a host bacterium different than *Escherichia coli*, and with a larger set of phages, will determine the broader applicability of this method.

With the addition of phage retron recombineering, retrons are solidifying their place in the toolbox of gene engineering techniques, which also includes restriction enzymes and CRISPR–Cas. It is noteworthy that all these techniques stem from immune systems that originally evolved to defend bacteria from phages. With the recent discovery that the bacterial pan-immune system contains many previously unknown

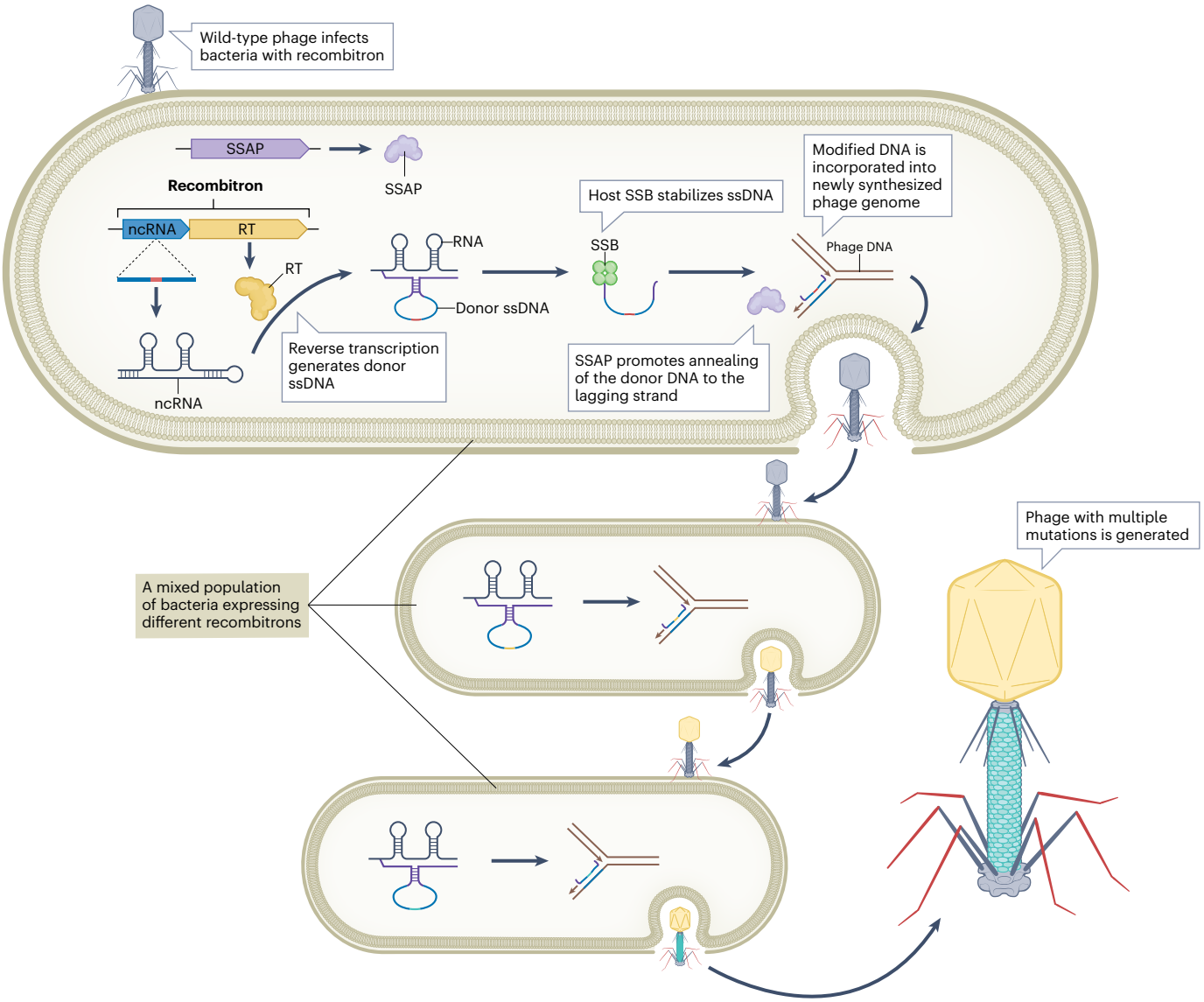

Fig. 1 | Phage retron recombineering. Recombitrons encode a modified noncoding RNA (ncRNA) and a reverse transcriptase (RT) that together produce a single-stranded DNA containing a desired edit flanked with sequences homologous to a locus in the phage genome. Combining a recombitron with a single-stranded annealing protein (SSAP), guided by an endogenous single-stranded DNA binding protein (SSB), allows efficient annealing of the donor DNA to the lagging strand of the replication fork in the replicating phage genome. A phage infecting a recombitron-expressing bacterium will be efficiently edited, and propagating phages through mixed bacterial cultures enables multiplexed editing of up to five edits in a single phage.

defense systems[9,10], it is expected that further research into bacterial immunity will yield more gene-editing tools in the future.

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Competing interests

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